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NOTES ON GEOGRAPHIC DISTRIBUTION

New distribution records and variation of the two common lowland salamanders *Bolitoglossa colonnea* (Dunn, 1924) and *B. lignicolor* (Peters, 1873) in Panama (Amphibia: Caudata: Plethodontidae)

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ABSTRACT: We revise the geographic distribution of two common lowland salamanders in western Panama. We close the widest gap in the known distribution of *Bolitoglossa colonnea* with a first record for the province of Veraguas and extend its known vertical distribution to a third life zone. For *B. lignicolor* we present additional localities in the province of Chiriquí and the first record from Comarca Ngöbe-Buglé that close the gap between extreme western Panama and the Azuero Peninsula. We present morphological, molecular, and coloration data for both species.

With 20 currently accepted species, the genus *Bolitoglossa* is the second most species-rich amphibian genus in Panama, after *Craugastor* (Hertz *et al.* 2012). Most of these salamanders inhabit the high mountains of the Talamancan mountain range in the Panama-Costa Rica border region. Only four known species occur in the lowlands of western Panama. Among these are, besides *B. biseriata*, Tanner 1962 and *B. schizodactyla*, Wake and Brame 1966, the two common species *B. colonnea* (Dunn 1924) and *B. lignicolor* (Peters 1873). *Bolitoglossa colonnea* is distributed primarily along the Caribbean slopes of the central mountain ranges of Costa Rica and Panama and into the Caribbean lowlands, including offshore islands. In contrast, *B. lignicolor* inhabits the Pacific drainage of southwestern Costa Rica and western Panama including the Isla de Coiba and the Azuero eninsula. However, there is an overlap in the distribution areas of both species in the humid lowlands of the Osa peninsula and the Golfo Dulce region of Costa Rica (McDiarmid and Savage 2005) and possibly marginally into extreme western Panama, where both species may be found sympatrically. These two species probably also meet in the Fortuna depression area, where the continental divide drops to less than 1000 m asl, interrupting the effect of the central mountain range as a barrier between the Caribbean and Pacific side climates. However, both species have not been recorded at the same site. Although both salamanders are frequently collected, documented collection sites are widely separated, creating a mosaic-like distribution pattern. We present new collection sites that close the largest gaps in the known distribution of both species for Panama. We further present coloration, morphology, and molecular data of the corresponding voucher specimens and discuss their variation.

All specimens were encountered during opportunistic searches at night, caught by hand, and preserved the day after capture. Collecting permits SE/A-30-08, SC/A-8-09, SC/A-28-09, SC/A-21-10, and SE/A-8-11, as well as the corresponding exportation permits were issued by the Dirección de Áreas Protegidas y Vida Silvestre, Autoridad Nacional del Ambiente - ANAM, Panama City. Voucher specimens have been deposited in the herpetological collection of the Senckenberg Forschungsinstitut und Naturmuseum (SMF), Frankfurt, Germany, as well as in the herpetological collection of the Universidad Autónoma de Chiriquí (Museo Herpetológico de Chiriquí - MHCH), Davíd, Chiriquí, Panama. Other collection acronyms follow Sabaj Pérez (2010), except for Círculo Herpetológico de Panamá (CH). Species identification was carried out employing the keys of Savage (2002) and Köhler (2011). Adult specimens were sexed by the presence of cloacal folds (females) or papillae (males). The capitalized colors and color codes (the latter in parentheses) provided for collected individuals are those of Smithe (1975–1981). Coordinates and elevation data were obtained using a Garmin GPS receiver with built-in barometric altimeter. All coordinates are in WGS 1984 datum, and elevations are rounded to the nearest 10 m. We obtained locality information for *Bolitoglossa colonnea* via HerpNet (http://www.herpnet.org) from the herpetological collections AMNH, FMNH, KU, MCZ, MVZ, and USNM, as well as from UP (Tejera and Dupuy 2003) and the web portal of the Círculo Herpetológico de Panamá (accessed through GBIF data portal, Datos de los Especimenes de Anfibios y Reptiles del Círculo Herpetológico de Panamá, http://ara.inbio.ac.cr/SSTN-IABIN/datasets/resource/37). The locality information for *B. lignicolor* was taken from ANSP, CAS, MVZ, and USNM via HerpNet, as well as from Brame and Wake (1963), Bauer *et al*. (1993), De la Riva (1997), and Cedeño *et al.* (2006). The map was created using ArcGIS 10. We obtained map layers from the map server of the Smithsonian Tropical Research Institute (STRI) (accessed

through http://mapserver.stri.si.edu/geonetwork/srv/en/main.home).

The following morphological measurements of voucher specimens were made with a caliper under a dissecting microscope: snout-vent length (SVL), from tip of snout to posterior angle of vent; tail length (TL), from posterior angle of vent to tip of tail; head length (HL), from tip of snout to gular fold; head width (HW), between angles of jaw; hind limb length (HLL), from groin to tip of longest toe; hand width (HAW), at greatest width; hind foot width (HFW), at greatest width. Tooth counts were made directly under a dissecting microscope: premaxillary teeth (PMT), maxillary teeth (MT), and vomerine teeth (VT).

A fragment of the mitochondrial 16S rRNA gene of selected voucher specimens was sequenced for comparison with sequences available on GenBank. Sequences were aligned with ClustalW (Larkin *et al.* 2007) using the default settings in Geneious (Drummond *et al.* 2010). We determined the Tamura 3-parameter model (Tamura 1992) as the best-fitting substitution model. Using MEGA5 (Tamura *et al.* 2011) we calculated p-distances and conducted Maximum Likelihood analyses (with 10000 bootstrap replicates). Using TCSv1.21 (Clement *et al.* 2000), we conducted a statistical parsimony network analysis, with gaps considered as a fifth character state. Because some haplotypes were not connected to each other with the 95% limit of probability of parsimony, we decreased the connection probability in both networks to a minimum of 90% resulting in a connection limit of 14 steps in our analysis.

A list of specimens examined, including corresponding GenBank accession numbers, is in the appendix.

***Bolitoglossa colonnea* (Dunn, 1924)**

Holotype: MCZ 9406, by original designation. Type locality: "La Loma, on trail from Chiriquicito to Boquete [Atlantic side], altitude about 2000 feet [610 m], Province of Bocas del Toro [today: Comarca Ngöbe-Buglé], Panama". (Figure 1, locality 1)

Diagnosis: *Bolitoglossa colonnea* is a moderate-sized species of salamander with fully webbed digits and only 0-11 maxillary teeth in adults, but up to 48 vomerine teeth. It is readily identified by a fleshy transverse ridge between the eyes.

Geographic distribution: An overview map that combines literature records with our own records is provided in Figure 1. Previous collection sites on the Caribbean side of the central mountain range extend over the humid lowlands and premontane elevations from northern Costa Rica to western Panama. The easternmost Caribbean records come from La Loma, Comarca Ngöbe-Buglé on the mainland (Figure 1, locality 1), and from Isla Escudo de Veraguas, Bocas del Toro (Figure 1, locality 2). On the Pacific side it is known from several Costa Rican sites around Golfo Dulce, and in Panama from three sites in the La Fortuna area (Figure 1, localities 3-5), and from the southern slopes of Cerro La Campana in central Panama (Figure 1, locality 6), the last of which is also the easternmost collection site for *B. colonnea*.

Our records from Cerro Negro, Río Chilagre, and Cerro Narices (Figure 1, localities 7-9) in the vicinity of Santa Fé, Veraguas represent the first records for that province and bridge the gap between Quebrada Bijau, La Fortuna Forest Reserve, Province of Chiriquí (Figure 1, locality 5) approximately 130 km west, and Altos de Campana, Province of Panamá (Figure 1, locality 6) approximately 130 km east of Santa Fé. The nearest collection site is the island Escudo de Veraguas, Province of Bocas del Toro (Figure 1, locality 2) about 85 km from Santa Fé in the Caribbean Sea. Our record from Río Changena, Province of Bocas del Toro, 1650 m (Figure 1, locality 10) extends the known vertical distribution of *Bolitoglossa colonnea* from 1250 m (Köhler 2011). This location expands the known habitat of *B. colonnea* from the Lowland and Premontane Life Zones to the Lower Montane Life Zone, specifically the Lower Montane Wet Forest (Holdridge 1967).

Variation: Coloration: Coloration of *Bolitoglossa colonnea* includes mostly shades of drab brown and beige, often with indistinct stripes and stippling. A compilation of color morphs is shown in Figure 2. Coloration in life of two specimens was recorded in the field as follows: SMF 94460 (Figure 2B). Dorsal ground color Cinnamon (39). Two Vandyke Brown (121) furcating lines start at interorbital transverse ridge, and meet posterior to head, thence continuing as single vertebral stripe to base of tail. Laterally Prout´s Brown (121 A) demarcated above by a fine Pale Pinkish Buff (121 D) line. The ventral surfaces mostly Pale Pinkish Buff (121 D) with fine Mikado Brown (121 C) longitudinal lines, except Vandyke Brown (221) gular region. SMF 94463 (Figure 2E). Dorsal ground color Chamois (123 D), irregularly mottled with Sepia (119). Distal 20% of tail grading into Pale Horn Color (92). Ventral surfaces Pale Horn Color (92) with fine mottling of Pratt´s Payne's Gray (88) and Sepia (119) spots and blotches, the larger ones grading into Dark Neutral Gray (83).

Morphology: Two males and seven females of *B. colonnea* were included in the morphological analysis. Ratios of TL, HL, HW, HLL, HAW and HFW in relation to SVL, as well as tooth counts for each specimen, are in Table 1. We calculated mean and standard deviation for males and females separately. Most specimens examined agree well with the morphological descriptions of other authors, but there is a discrepancy in some tooth counts. Savage (2002) gave the number of maxillary teeth as 0-6, while in our sample many females have more than six and up to eleven maxillary teeth. Further, a male specimen (MHCH 2600) from Río Chilagre, Veraguas (Figure 1, locality 8) raises the known maximum of vomerine teeth of this species from 36 (Savage 2002) to 48. Figure 3 illustrates the VT/SVL ratio for males and females from eastern and western localities separately. The highest vomerine tooth counts were recorded in specimens from eastern localities. On average, males seem to have longer tails and more premaxillary teeth than females, although our sample of males is too small to confirm this (Table 1).

Molecular genetics: We compared 16S mtDNA sequences of two specimens (SMF 94460–61) collected at Río Changena and Río Clarito (Figure 1, localities 10 and 11) in highland Bocas del Toro, to two specimens on GenBank, one from Finca de Enrique Quintero, Río Changuinola (Figure 1, locality 12) in lowland Bocas del Toro and one from La Fortuna Forest Reserve (Figure 1, near Locality 4, exact locality unknown). We chose the sister species *Bolitoglossa schizodactyla* as an outgroup (Figure 4). Our

two specimens (SMF 94460–61.) from higher elevations of Bocas del Toro share the same haplotype, they differ by 1.9% from one specimen on GenBank (CH 6526) that was collected at lower elevation at Río Changuinola (Figure 1, locality 12; Table 2). All three specimens have been connected in a haplotype network (Figure 4), with a calculation of 10 unsampled haplotypes between our two specimens from upland Bocas del Toro and the one from the lowlands. The fourth specimen (AY526119, no voucher) from Hornito, La Fortuna Forest Reserve, Chiriquí (Figure 1, locality 4), referred to as *B. colonnea*, has not been connected to the haplotype network and shows a p-distance of 4.2 to 5.4% to the specimens collected in Bocas del Toro, while its distance to the outgroup is only 5.2%.

Remarks: There is no other salamander species in lower Central America that is so easily recognized, owing to its conspicuous and unique fleshy interorbital ridge. Nevertheless, there is variation in morphology (Table 1; Figures 2 and 3) and genetics (Table 2; Figure 4). We found the highest count of vomerine teeth in two specimens from Río Chilagre, Veraguas. This difference is considerable between the two males (Figure 3), from widely separated localities, one (SMF 94460, Figure 2B) from Río Changena, Bocas del Toro (Figure 1, locality 10) in extreme western Panama on the Caribbean slopes of the Talamancan mountain range, the other (MHCH 2600, Figure 2F) from Río Chilagre, Veraguas (Figure 1, locality 8) on the eastern end of the central mountain range. Although both specimens are about the same size, the male from Río Chilagre (MHCH 2600) has 48 vomerine teeth, 31 more than the male from Río Changena (SMF 94460). Savage (2002) gave a maximum of 36 vomerine teeth for adults of *B. colonnea*. In females, the specimen (SMF 94461, Figure 2A) from Río Clarito, Bocas del Toro (Figure 1, locality 11), the largest in our sample, has 11 vomerine teeth fewer than the considerably smaller female (SMF 94463, Figure 2E) from Río Chilagre, Veraguas (Figure 1, locality 8), which has 34 vomerine teeth. Another subadult female (SMF 85066, Figure 2C) specimen from Isla Colón, Bocas del Toro (Figure 1, locality 13), the smallest in our sample, has one vomerine tooth fewer than the next largest female (SMF 94464, Figure 2D) from Cerro Negro, Veraguas (Figure 1, locality 7). Our data suggest that

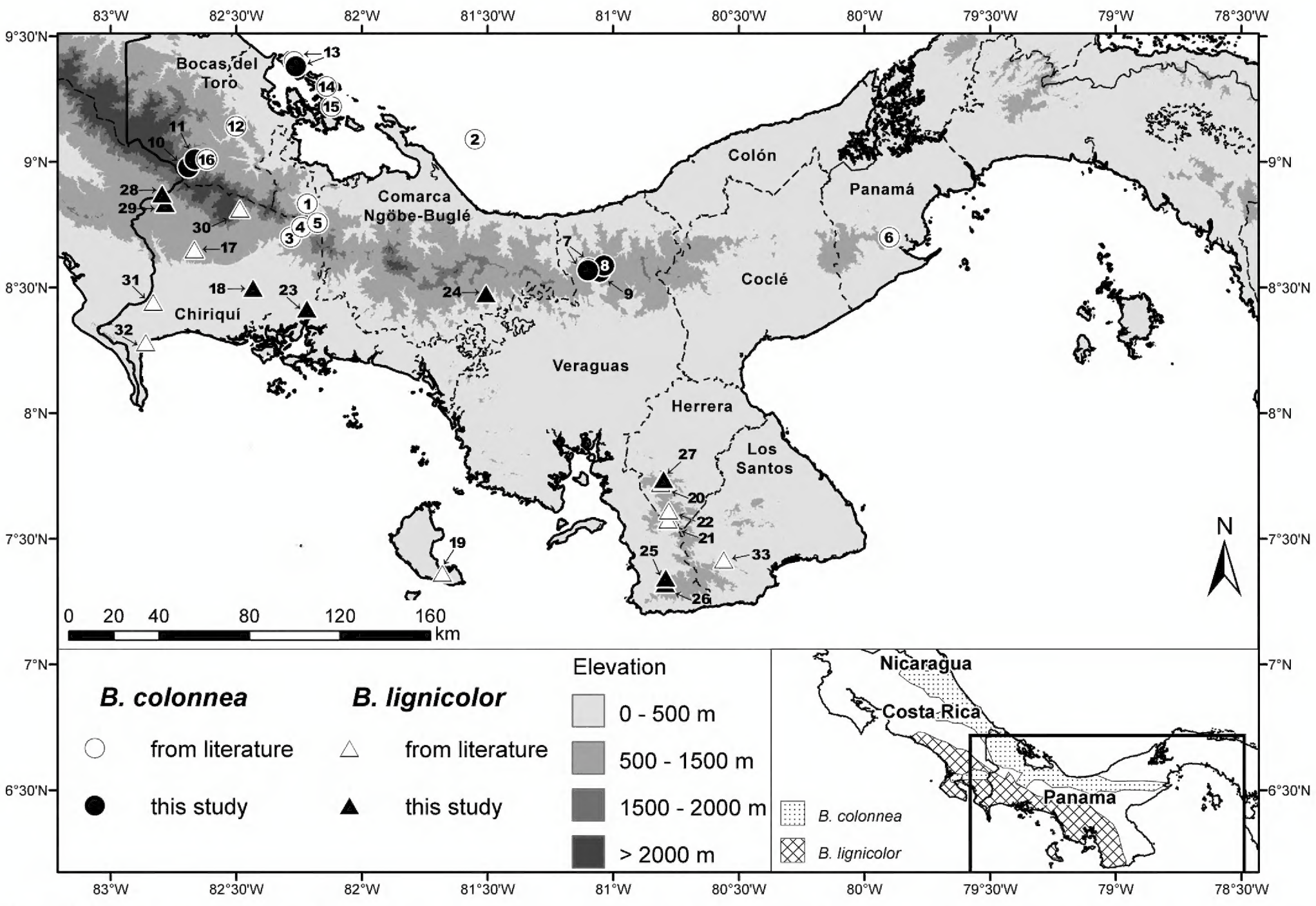

Figure 1. Map showing collection sites of *Bolitoglossa colonnea* and *B. lignicolor* in Panama (the overall distribution is shown in the overview map in lower right corner). Solid lines represent coastal lines and national borders, dashed lines represent provincial borders. Numbers on the map refer to the following locations (One symbol may represent several specimens from different localities close to each other; see Appendix for details): 01 La Loma, Comarca Ngöbe-Buglé (type locality of *B. colonnea*); 02 Escudo de Veraguas, Bocas del Toro; 03 Los Planes, Chiriquí; 04 Hornito, Chiriquí; 05 Quebrada Bijau, La Fortuna Forest Reserve, Chiriquí; 06 Cerro La Campana, Panamá; 07 Cerro Negro, Veraguas; 08 Río Chilagre, Veraguas; 09 Cerro Narices, Veraguas; 10 Río Changena, Bocas del Toro; 11 Río Clarito, Bocas del Toro; 12 Finca de Enrique Quintero, Río Changuinola, Bocas del Toro; 13 Isla Colón, Bocas del Toro; 14 Bastimentos, Bocas del Toro; 15 Isla Popa, Bocas del Toro; 16 Río Claro, Bocas del Toro; 17 Camarón Arriba, Chiriquí (type locality of *B. lignicolor*); 18 City of Davíd and vicinity, Chiriquí; 19 Isla de Coiba, Veraguas; 20 Montuoso Forest Reserve, Chepo and Las Minas Districts, Herrera (MVZ site); 21 Cerro Manguillo, Veraguas; 22 Tiger Ridge Camp, Herrera; 23 Meseta de Chorcha, Chiriquí; 24 Alto Tólica, Comarca Ngöbe-Buglé; 25 Cerro Hoya, Veraguas (Locality A); 26 Cerro Hoya, Veraguas (Locality B); 27 Montuoso Forest Reserve, Herrera (this study); 28 Finca C.A.S.A., north of Río Sereno, Chiriquí; 29 Santa Clara, Chiriquí; 30 Finca Lérida, Chiriquí, 31 Progreso, Chiriquí; 32 Puerto Armuelles, Chiriquí; 33 La Tronosa Forest Reserve, Los Santos.

specimens from the eastern portion of the range tend to have more vomerine teeth than those from the west, even considering that the number of teeth increases with size and age (Figure 3).

We also observed genetic differences in 16S gene sequences. The distance between the specimen (AY526119, no voucher) from Hornito, La Fortuna Forest Reserve, Chiriquí (Figure 1, locality 4) and the others (SMF 94460–61; CH 6526) from Bocas del Toro (Figure 1, localities 10-12) is large enough to suggest that two different species may be involved. Unfortunately, there is no voucher specimen corresponding to the sequence of the specimen from Hornito, La Fortuna Forest Reserve (Parra-Olea *et al.* 2004). During our field work in the La Fortuna Forest Reserve we could not obtain specimens of *B. colonnea* or any other species that may resemble *B. colonnea.* This is a subject for future study.

***Bolitoglossa lignicolor* (Peters 1873)**

Syntypes: ZMB 7736 (2 specimens); ZMB 7736A

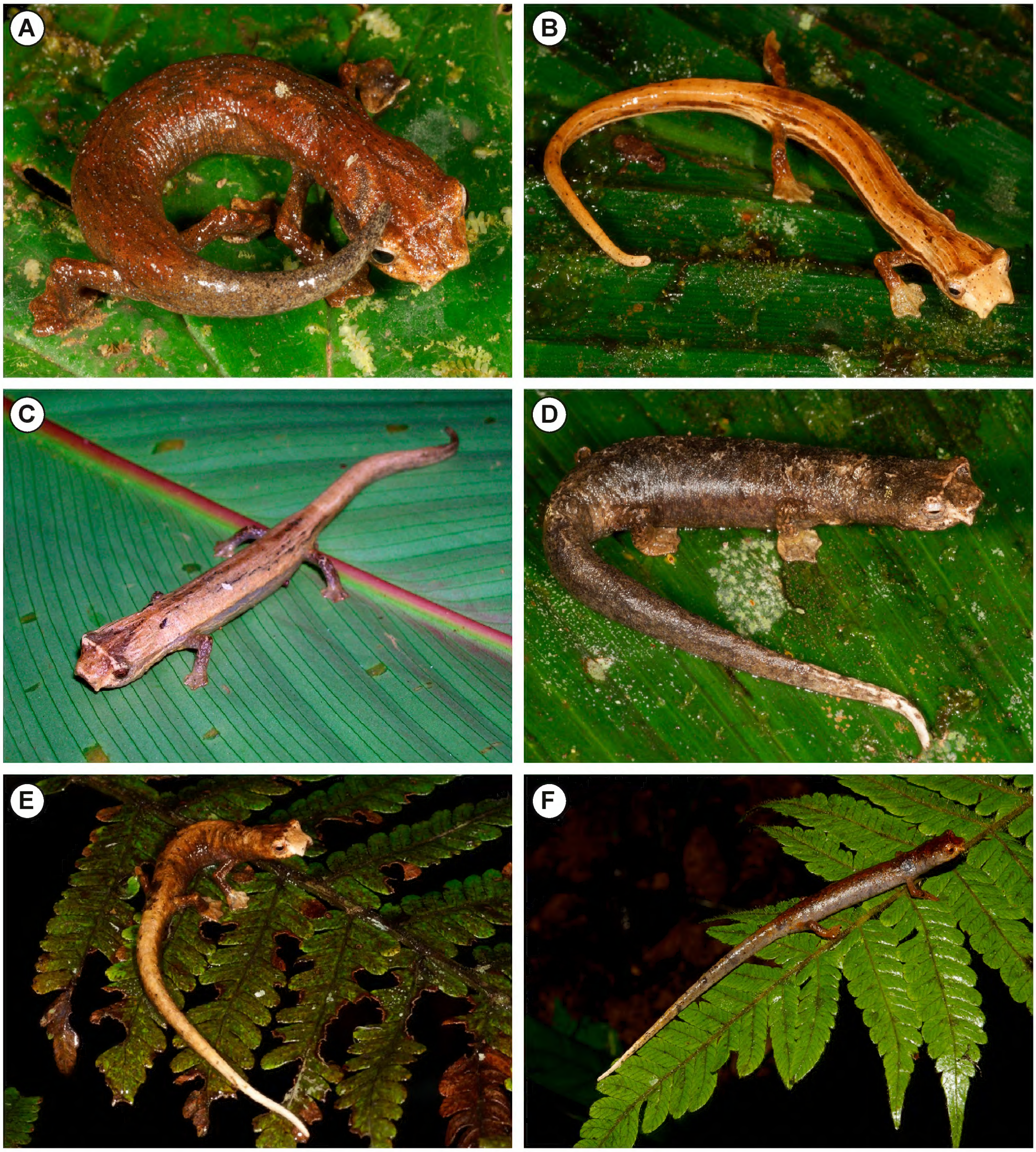

Figure 2. Selected specimens of *Bolitoglossa colonnea*: A) Female (SMF 94461) from Río Clarito, Bocas del Toro (Figure 1, locality 11); B) Male (SMF 94460) from Río Changena, Bocas del Toro (Figure 1, locality 10); C) SMF 85066 from Isla Colón, Bocas del Toro (Figure 1, locality 13), (Photo courtesy of J. Sunyer); D) Female (SMF 94464) from Cerro Negro, Veraguas (Figure 1, locality 7); E) Female (SMF 94463) from Río Chilagre, Veraguas; F) Male (MHCH 2600) from Río Chilagre, Veraguas (Figure 1, locality 8).

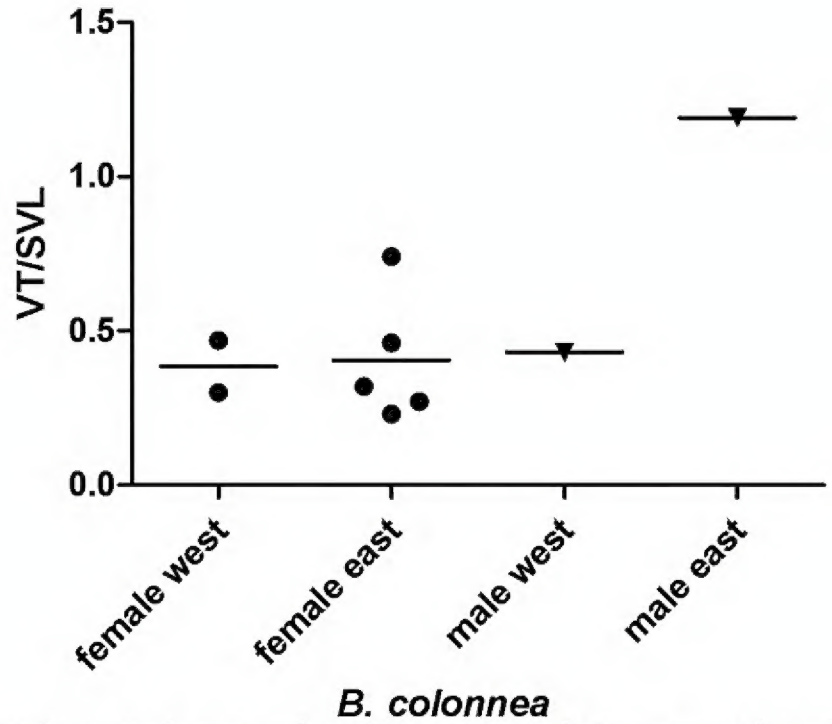

Figure 3. Vertical scatter plot of vomerine teeth/snout-vent-length ratio for *Bolitoglossa colonnea*. Males and females from eastern and western localities are shown separately. Horizontal bars indicate mean values.

designated lectotype by Bauer *et al*. 1993. Type locality: "Chiriqui", Panama; corrected by Bauer *et al.* (1993) to "Camarón, Provinz Chiriqui", Panama (Figure 1, locality 17, see remarks).

Diagnosis: *Bolitoglossa lignicolor* is a relatively large and robust species with fully webbed hands and feet. It usually has a light brown dorsum, while the venter is dark brown.

Geographic distribution: An overview map that combines literature records with our own records is given in Figure 1. Previously, *Bolitoglossa lignicolor* has been collected at various sites in southwestern Costa Rica, along the Pacific coast from Manuel Antonio National Park to the Osa Peninsula and the Golfo Dulce region, into extreme western Panama approximately as far as the vicinity of the city of Davíd (Lotzkat and Hertz 2011; Batista and Ponce 2011; Figure 1, locality 18). Brame and Wake (1963) listed collection sites along the Caribbean drainage of northern

Table 1. Morphological characters and tooth counts for specimens of *Bolitoglossa colonnea* from Panama.

NUMBER	SEX	SVL	SVL/TL	SVL/HL	SVL/HW	SVL/HLL	SVL/HAW	SVL/HFW	PMT	MT	VT
SMF89920	female	47.4	1.1	4.2	6.3	4.6	11.3	8.3	3	10	11
SMF89921	female	47.2	1.0	4.0	6.3	4.6	11.8	8.6	3	9	15
SMF89919	female	43.7	0.9	3.8	6.0	4.5	11.5	9.3	2	9	20
SMF94463	female	46.2	0.9	4.0	6.1	5.0	12.8	10.0	3	11	34
SMF94464	female	40.9	1.1	4.0	5.6	4.3	11.1	10.0	2	7	11
SMF94461	female	49.0	tail lost	4.3	6.1	4.9	12.0	9.6	0	8	23
SMF85066	female	33.0	1.4	4.4	6.1	5.3	13.2	12.2	2	0	10
MHCH 2600	male	40.3	0.9	4.2	6.9	4.1	11.5	9.4	5	0	48
SMF94460	male	40.0	1.0	3.9	6.2	4.3	11.1	8.7	6	2	17
Mean±SD	female	43.9±5.51	1.1±0.18	4.1±0.20	6.1±0.24	4.7±0.36	11.9±0.80	9.7±1.28	2.1±1.07	7.7±3.64	17.7±8.71
Mean±SD	male	40.2±0.21	0.9±0.03	4.1±0.02	6.6±0.56	4.2±0.20	11.3±0.29	9.0±0.48	5.5±0.71	1.0±1.41	32.5±21.92

Table 2. Estimates of evolutionary divergence between sequences of specimens of *Bolitoglossa colonnea. Bolitoglossa schizodactyla* has been chosen as an outgroup. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Tamura 3-parameter model.

	1	2	3	4	5	6
1– *B. colonnea* SMF94460 (PA: Bocas del Toro: Río Changena)	-					
2– *B. colonnea* SMF94461 (PA: Bocas del Toro: Río Clarito)	0.000	-				
3– *B. colonnea* CH6526 (PA: Bocas del Toro: Río Changuinola)	0.019	0.019	-			
4– *B. colonnea* AY526119 (PA: Chiriquí: La Fortuna)	0.042	0.042	0.054	-		
5– *B. schizodactyla* FJ784482 (PA: Coclé: El Copé)	0.061	0.061	0.063	0.052	-	
6– *B. schizodactyla* AY526133 (PA: Coclé)	0.061	0.061	0.063	0.052	0.000	-

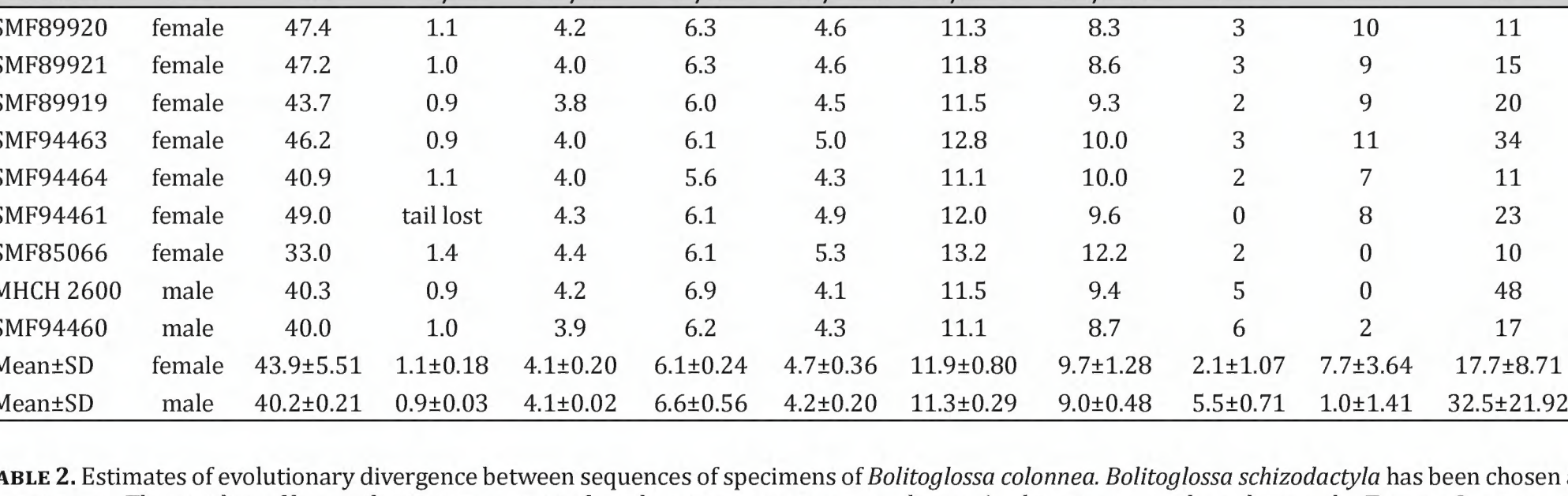

Figure 4. Maximum Likelihood consensus tree of 16S mtDNA of selected specimens of *Bolitoglossa colonnea*, with *B. schizodactyla* as an outgroup, and the corresponding haplotype network. Specimen labels refer to collection number or, if there is no collection number available, to GenBank accession number. The upper two specimens have been sequenced for this study. Numbers in rectangles refer to the respective collection site in Figure 1. Other collection sites are those denoted on GenBank: Coclé = Parque Nacional G. D. Omar Torrijos H., El Copé, Distrito La Pintada, Cocle Province, 800 m elevation.

Costa Rica (AMNH 11725 and KU 66164) that are in fact based on specimens of *B. alvaradoi*. Additional collection sites in Panama are Coiba Island (De la Riva 1997; Figure 1, locality 19) and the Azuero Peninsula (Brame and Wake 1963; Figure 1, localities 20–22 and Cedeño *et al.* 2006; Figure 1, locality 33), approximately 200 km to the east. Brame and Wake (1963) believed "Tiger Ridge Camp" and "Cerro Mangillo" to be located in the province of Los Santos. Actually, the collection data on the ANSP specimens read "Veraguas prov., Cerro Mangillo, 2800 ft." and "Veraguas prov.?, Tiger Ridge Camp 2600 ft." (Ned Gilmore, ANSP pers. comm. 2012). However, none of these place names is traceable on recent maps. There is only one mountain named Cerro Manguillo (or Cerro Manglillo) on Azuero Peninsula, located near the present day province triangle Veraguas, Herrera, and Los Santos. We assume that E.R. Dunn, who collected the specimens, started his expedition to Cerro Manguillo (Figure 1, locality 21) from the Veraguas side and was not sure if he was still in Veraguas when he reached Tiger Ridge Camp, which he

Figure 5. Selected specimens of *Bolitoglossa lignicolor*: A) Male (SMF 85059) from Finca C.A.S.A., Chiriquí (Figure 1, locality 28); B) Female (SMF 94458) from Santa Clara, Chiriquí (Figure 1, locality 29); C) Female (SMF 89803) from near City of Davíd, Chiriquí (Figure 1, locality 18); D) Female (SMF 85065) from Meseta de Chorcha, Chiriquí (Figure 1, locality 23); E) Male (SMF 94459) from Alto Tólica, Comarca Ngöbe-Buglé (Figure 1, locality 24); F) Male (SMF 91994) from Cerro Hoya, Veraguas (Figure 1, locality 26).

expressed by the question mark. We suspect Tiger Ridge Camp (Figure 1, locality 22) has been on the crest above Quebrada El Tigre, about 4 km north of Cerro Manguillo in Herrera province. It is the only place that is name-wise linked to Tiger Ridge, as well as at the stated elevation. This view is supported by the fact that ANSP specimens with interjacent numbers were collected at Macaraquito Camp which is situated between these two places. The documented vertical distribution ranges from sea level to approximately 1200 m asl.

We present two more records from the Pacific slopes of the central mountain range that fill the gap in the distribution of *Bolitoglossa lignicolor*. The first new locality is Meseta de Chorcha, Chiriquí, about 25 km east of the city of Davíd (Figure 1, locality 23). The second is Alto Tólica, Comarca Ngöbe-Buglé (Figure 1, locality 24), about 80 km east of Meseta de Chorcha, also representing the first record for the Comarca Ngöbe-Buglé. Moreover, we present additional localities on the Azuero Peninsula (Figure 1, localities 25 and 26) and confirm a previous collection site at Montuoso Forest Reserve (Figure 1, localities 20 and 27).

Variation: Coloration: The usual coloration pattern is a broad, light-colored dorsal band, which may be light tan, beige or reddish, on darker, mostly chocolate brown ground coloration. However, the light dorsal coloration may be broken up into blotches and even smaller spots. In contrast, some individuals are predominantly light colored with darker mottling. Color photographs of selected specimens are in Figure 5. The coloration in life of three specimens has been recorded in the field as follows: SMF 91996 (not pictured). Dorsum Tawny (38) with Raw Umber (223) streaks. Iris Verona Brown (223 B). SMF 91994 (Figure 5F). Dorsal ground color Sepia (119) with dirty white stipples grading into Tawny Olive (223 D) on tail. Ventral surfaces the same as dorsal surfaces, but with finer and less dense stippling. Iris Drab Gray (119 D), peripherally Mars Brown (223 A). SMF 94458 (Figure 5B). Dorsal ground color Clay Color (26) with longitudinal, broken lines of Sepia (119) and Cream Color (54). Ventral ground color Dark Brownish Olive (129) powdered with dirty white spots. Limbs Dark Brownish Olive (129) dorsally suffused with Clay Color (26).

Morphology: Six males and 12 females of *Bolitoglossa lignicolor* were included in the morphological analysis. Ratios of TL, HL, HW, HLL, HAW and HFW in relation to SVL, as well as tooth counts, for each specimen are in Table 3. There is not much variation within sexes in our sample, while variation between sexes is evident. As in *B. colonnea*, males have longer tails in relation to their snout-vent length. We also observed slightly higher tooth counts for males. In contrast, Brame and Wake (1963) stated that females of *B. lignicolor* have more vomerine teeth, because the number of teeth increases with age and size and females grow bigger than males. However, in our sample males have higher vomerine tooth counts, and males in our sample are on average larger than females,

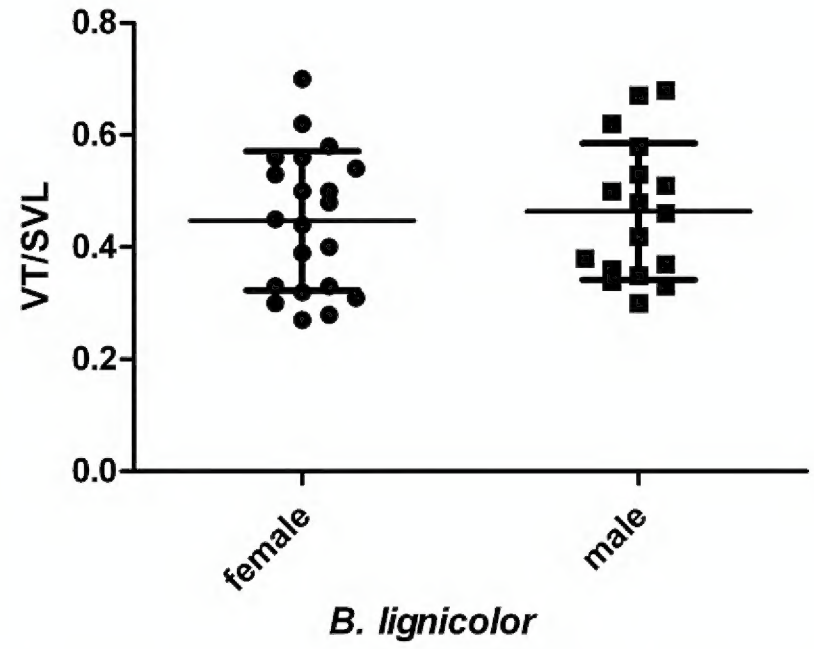

FIGURE 6. Vertical scatter plot of vomerine teeth/snout-vent-length ratio for *Bolitoglossa lignicolor*. Males and females kept separated. Centered horizontal bars indicate mean values; upper and lower horizontal bars standard deviation.

TABLE 3. Morphological characters and tooth counts for specimens of *Bolitoglossa lignicolor* from Panama.

NUMBER	SEX	SVL	SVL/TL	SVL/HL	SVL/HW	SVL/HLL	SVL/HAW	SVL/HFW	PMT	MT	VT
SMF94458	female	50.2	1.4	4.1	6.3	5.1	12.2	10.7	4	33	35
SMF89802	female	64.1	1.1	4.7	6.7	5.1	12.8	10.7	0	35	28
MHCH 2601	female	44.6	1.2	4.5	6.7	5.3	13.9	9.9	0	24	25
SMF89803	female	63.7	1.3	5.1	6.7	4.6	13.8	10.4	0	39	21
MHCH 2602	female	46.0	1.2	4.2	6.4	4.9	13.1	10.2	1	28	23
SMF85063	female	30.0	1.3	3.6	5.8	4.3	14.3	11.1	0	13	10
SMF85060	female	30.1	1.5	4.0	5.7	6.0	14.3	10.8	0	12	17
SMF85065	female	36.1	1.3	4.1	6.0	5.4	14.4	12.9	0	18	10
SMF91995	female	32.4	1.4	4.0	5.7	5.5	15.4	12.0	0	20	13
SMF91996	female	69.2	1.4	4.5	6.6	5.6	13.6	11.2	1	40	19
SMF91997	female	56.7	tail lost	4.2	6.7	5.5	13.5	10.5	4	39	17
SMF92001	female	30.1	tail lost	3.8	5.4	5.3	14.3	11.6	0	13	15
SMF94459	male	67.0	1.1	4.2	6.4	6.1	13.4	10.6	4	51	25
SMF85059	male	52.2	1.0	4.5	6.2	5.0	12.4	9.8	5	35	35
SMF91998	male	53.4	1.1	4.0	6.4	5.2	14.1	11.6	3	38	19
SMF91994	male	57.3	0.9	4.2	6.4	5.2	12.7	10.1	4	47	19
SMF91999	male	47.0	1.0	4.2	6.4	5.3	13.4	10.9	3	31	16
SMF92000	male	45.4	1.1	4.1	6.1	4.8	12.6	10.1	4	22	31
Mean±SD	females	46.01±14.64	1.33±0.12	4.23±0.40	6.21±0.49	5.22±0.46	13.82±0.84	10.99±0.83	0.83±1.53	26.17±10.89	19.42±7.46
Mean±SD	males	53.72±7.82	1.03±0.05	4.19±0.17	6.30±0.14	5.26±0.44	13.11±0.62	10.53±0.67	3.83±0.75	37.33±10.6	24.17±7.55

although our specimens are generally smaller than the ones examined by Brame and Wake (1963). Nonetheless, when combining the data of Brame and Wake (1963, Table 1, p. 292 excluding AMNH 11725 and KU 66164) and our data in a scatter plot, and factoring in SVL, VT/SVL ratio remains slightly higher for males, even though females attain larger maximum sizes than males (Figure 6).

Molecular genetics: We compared 16S mtDNA of six specimens (SMF 89803, 91994, 91996-7, 94459, and MHCH 2602) to a specimen assigned to *Bolitoglossa lignicolor* (MVZ 11132) from Costa Rica that is on Genbank. As outgroups, we chose two related species, one specimen of *B. mexicana* from Mexico and one of *B. mombachoensis* from Nicaragua (Figure 7). There is comparatively little intraspecific variation in the 16S gene of *B. lignicolor*. The highest p-distance of about 2% has been calculated between specimens from Panama and the one from Costa Rica (Table 4).

Remarks: The type locality of *Bolitoglossa lignicolor* is not clear. Bauer *et al.* (1993), when examining the type material, found a label in the jar giving the collection site of the holotype as "Camarón, Province of Chiriquí". Camarón, Spanish for "shrimp", has been copied incorrectly by several authors (*e.g.,* Frank and Ramus 1995, Frost 2011, Köhler 2011) as "Camron". We examined several maps and found three sites called Camarón in Chiriquí, which all are situated within the assumed distribution area of *B. lignicolor*. One "Camarón" is on the banks of the Río Tabasará, near the provincial boundary with Veraguas in the district of Tolé. Further, we found two "Camarón Arriba", the first being located in what is now the Comarca Ngöbe-Buglé, but which had been part of Chiriquí before 1997, approximately 14 km north-east of San Lorenzo, and a second "Camarón Arriba" in the district of Bugaba. Although the collector of the type material, entomologist H. Ribbe, did not record an itinerary, we suspect the type locality to be the second Camarón Arriba, about 10 km north of the city of Bugaba (Figure 1, locality 17). A few years later, G.C. Champion collected insects for F.D. Godman, who mentioned this place in his notes (Godman 1915). This location is well within the known distribution range of *B. lignicolor* and was quite accessible even in the 19th century, as it is not far from the road to Costa Rica. This evidence gives us reason to restrict the type locality to "Camarón Arriba, District of Bugaba, Province of Chiriquí" (Figure 1, Locality 17).

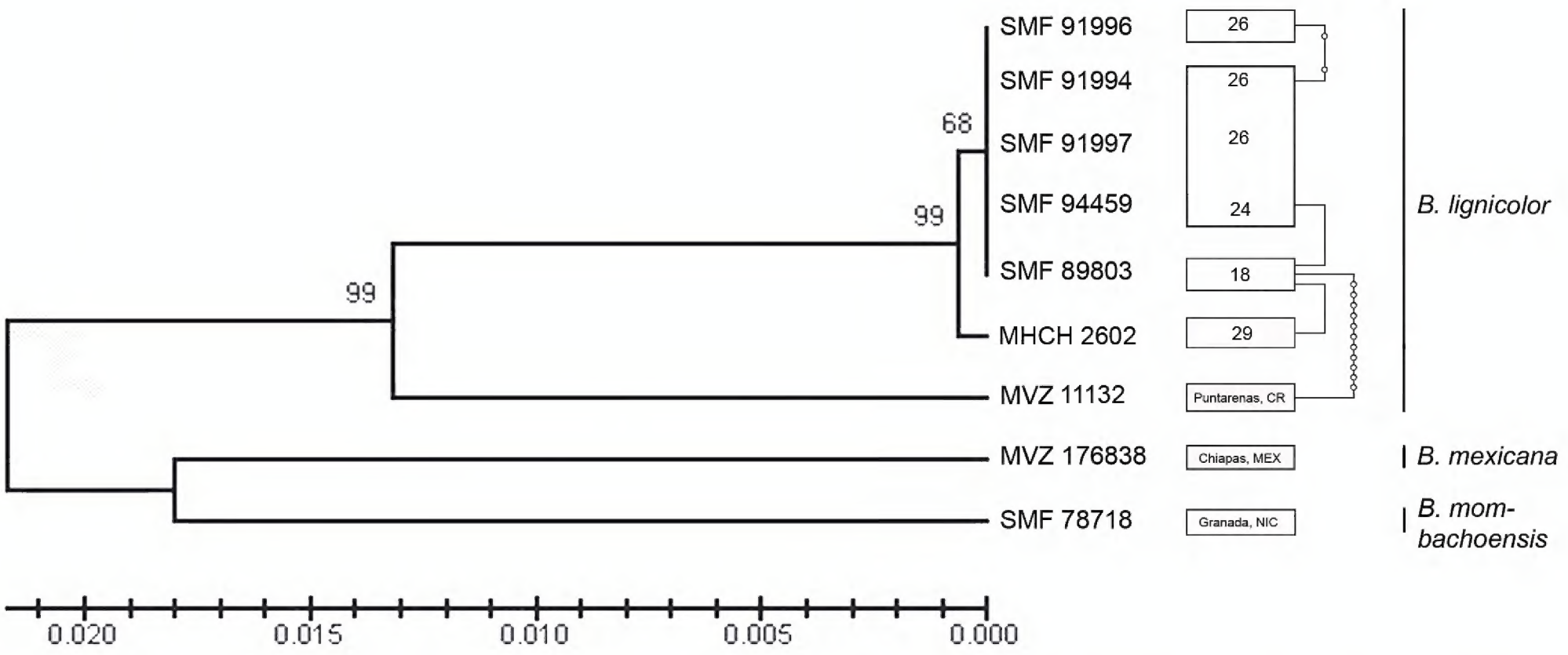

Figure 7. Maximum Likelihood consensus tree of the 16S mtDNA of selected specimens of *Bolitoglossa lignicolor*, with *B. mexicana* and *B. mombachoensis* as outgroups, and the corresponding haplotype network. The upper six specimens have been sequenced for this study. Specimen labels refer to collection numbers. Numbers in rectangles refer to the respective collection site in Figure 1. Other collection sites are those denoted on GenBank: Puntarenas, CR = Province of Puntarenas, Costa Rica; Chiapas, MEX = Chiapas State, Mexico; Granada, NIC= Department of Granada, Nicaragua.

Table 4. Estimates of evolutionary divergence between sequences of specimens of *Bolitoglossa lignicolor*. *Bolitoglossa mexicana* and *B. mombachoensis* have been chosen as outgroups. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Tamura 3-parameter model.

	1	2	3	4	5	6	7	8	9
1– *B. lignicolor* SMF94459 (PA: Ngöbe-Buglé: Alto Tólica)	-								
2– *B. lignicolor* SMF91997 (PA: Veraguas: Cerro Hoya)	0.000	-							
3– *B. lignicolor* SMF89803 (PA: Los Algarrobos: Chiriqui)	0.000	0.000	-						
4– *B. lignicolor* SMF91996 (PA: Veraguas: Cerro Hoya)	0.000	0.000	0.000	-					
5– *B. lignicolor* SMF91994 (PA: Veraguas: Cerro Hoya)	0.000	0.000	0.000	0.000	-				
6– *B. lignicolor* MHCH 2602 (PA: Chiriquí: Santa Clara)	0.002	0.002	0.002	0.002	0.002	-			
7– *B. lignicolor* MVZS11132 (CR: Puntarenas: Buenos Aires)	0.020	0.020	0.020	0.020	0.020	0.022	-		
8– *B. mexicana* MVZ176838 (MEX: Chiapas)	0.041	0.041	0.041	0.045	0.041	0.039	0.043	-	
9– *B. mombachoensis* SMF78718 (NIC: Granada)	0.030	0.030	0.030	0.034	0.030	0.032	0.032	0.026	-

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Literature Cited

Batista, A. and M. Ponce. 2011 "2010". Anfibios y reptiles (Amphibia: Anura, Caudata; Reptilia: Crocodylia, Squamata, Testunides) del Jardín Botánico de la Unversidad Autónoma de Chiriquí. *Puente Biológico* 3: 111-125.

Bauer, A.M., D.A. Good and R. Günther. 1993. An annotated type catalogue of the caecilians and salamanders (Amphibia: Gymnophiona and Caudata) in the Zoological Museum, Berlin. *Mitteilungen aus dem Zoologischen Museum in Berlin* 69: 285-306

Brame, A.H. and D.B. Wake. 1963. Redescription of the plethodontid salamander *Bolitoglossa lignicolor* (Peters), with remarks on the status of *B. palustris* Taylor. *Proceedings of the Biological Society of Washington* 76: 289-296.

Cedeño, J., V. Martínez Cortés and H. Fossatti. 2006. Anfibios en la Reserva Forestal la Tronosa: Diversidad y estado de conservación. *Tecnociencia* 8(2): 101-114.

Clement, M., D. Posada and K. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology Notes,* 9: 1657-1660.

De la Riva, I. 1997. La fauna de anfibios del Parque Nacional de Coiba (Panamá): Composición y biogeografía; p. 419-432 *In* S. Castroviejo (ed.). *Flora y Fauna del Parque nacional de Coiba (Panamá)*. Madrid: AECI.

Drummond, A.J., B. Ashton, M. Cheung, J. Heled, M. Kearse, R. Moir, S. Stones-Havas, T. Thierer and A. Wilson. 2010. *Geneious v.4.8.5.* Accessible at www.geneious.com. Captured on 10 November 2011.

Dunn, E.R. 1924. New salamanders of the genus *Oedipus* with a synoptical key. *Field Museum of Natural History Publication. Zoological Series* 12: 95-100.

Frank, N. and E. Ramus. 1995. *Complete Guide to Scientific and Common Names of Amphibians and Reptiles of the World*. Pottsville: N. G. Publishing Inc. 377 p.

Frost, D.R. 2011. *Amphibian Species of the World: an Online Reference. Version 5.5 (31 January, 2011).* Electronic Database accessible at http://research.amnh.org/vz/herpetology/amphibia/. American Museum of Natural History, New York, USA. Captured on 08 July 2012.

Godman, F.D. 1915. Biologia Centrali-Americana. Zoology, Botany and Archaeology. Introductory Volume; *In* F.D. Godman and O. Salvin (ed.) (1879-1904). *Biologia Centrali-Americana. Zoology, Botani and Archaeology*. London: Taylor & Francis. 208 p.

Hertz, A., S. Lotzkat, A.R. Carrizo Diaz, M. Ponce, G. Köhler and B. Streit. 2012. Field notes on findings of threatened amphibian species in the central mountain range of western Panama. *Amphibian and Reptile Conservation* 6: 9-30.

Holdridge, L.R. 1967. *Life zone ecology.* San Jose: Tropical Science Center. 206 p.

Köhler, G. 2011. *Amphibians of Central America*. Offenbach: Herpeton. 400 p.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins. 2007. ClustalW and ClustalX version 2. *Bioinformatics* 23(21): 2947-2948.

Lotzkat, S. and A. Hertz. 2011 "2010". La herpetofauna (Amphibia: Anura, Caudata; Reptilia: Crocodylia, Squamata, Testunides) de Los Algarrobos, Chiriquí, occidente de Panamá. *Puente Biológico* 3: 89-99.

McDiarmid, R.W. and J.M. Savage. 2005. The herpetofauna of the Rincón area, Peninsula de Osa, Costa Rica, a Central American lowland evergreen forest site; p. 366-427 *In* M.A. Donnelly, B.I. Crother, C. Guyer, M.H. Wake and M.E. White (ed.). *Ecology and evolution in the tropics: a herpetological perspective*. Chicago: The University of Chicago Press.

Parra-Olea, G., M. García-París and D.B. Wake. 2004. Molecular diversification of salamanders of the tropical American genus *Bolitoglossa* (Caudata: Plethodontidae) and its evolutionary and biogeographical implications. *Biological Journal of the Linnean Society London* 81: 325-346.

Sabaj Pérez, M.H. 2010: *Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference. Verson 1.5 (4 Oct 2010).* American Society of Ichthyologists and Herpetologists. Accessible online at http://www.asih.org. Captured on 10 December 2010.

Savage, J.M. 2002. *The amphibians and reptiles of Costa Rica. A herpetofauna between two continents, between two seas.* Chicago: The University of Chicago Press. xx+934 p.

Smithe, F.B. 1975-1981. *Naturalist's color guide. Part I. Color guide. 182 color swatches.* New York: American Museum of Natural History.

Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* 9: 678-687

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.

Tejera, V.H. and O.A. Dupuy 2003. *Anfibios del Museo de Vertebrados de la Universidad de Panamá, catálogo*. Panamá: Museo de Vertebrados de la Universidad de Panamá: 187 p.

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Appendix. Specimens examined (GenBank accession number of sequenced specimens in parentheses behind collection number; number in parentheses behind coordinates refers to locality in Figure 1):
Bolitoglossa colonnea: Panama: Bocas del Toro: Isla Colón, 9°22'48" N, 82°15'45" W, 30 m: SMF 85066; Río Clarito, 9°00'35" N, 82°39'50" W (13), 1250 m: SMF 94461 (JX434645); Río Changena, 8°58'44" N, 82°41'24" W (10), 1650 m: SMF 94460 (JX434644). Veraguas: Parque Nacional Santa Fé: Cerro Negro, 8°34'05" N, 81°06'02" W (7), 700 m: SMF 94464; 8°34'37" N, 81°05'50" W (7), 800 m: SMF 89920-21; Río Chilagre, 8°35'14" N, 81°02'11" W (8), 460 m: SMF 94463, MHCH 2600; Cerro Narices, 8°33'47" N, 81°03'12" W (9), 700 m: SMF 89919. ***Bolitoglossa lignicolor***: Panama: Chiriquí: Finca C.A.S.A., 8 km NE of Río Sereno, 8°52'17" N, 82°47'43" W (28), 1210 m: SMF 85059-60; Santa Clara, Finca Caballero, 8°49'59" N, 82°46'57" W (29), 1200 m: SMF 94458, MHCH 2602 (JX434638); Los Algarrobos, 8 km N of Davíd, Casa de la Alemana, 8°29'47" N, 82°25'59" W (18), 125 m: SMF 89802, 89803 (JX434642), MHCH 2601. Comarca Ngöbe-Buglé: Alto Tólica, 8°28'29" N, 81°30'20" W (24), 1050 m: SMF 94459 (JX434639). Veraguas: Cerro Hoya, 7°20'33" N, 80°47'26" W (25), 210 m: SMF 91995; 7°19'12" N, 80°47'24" W (26), 930-960 m: SMF 91994 (JX434641), SMF 91996 (JX434643), 91997 (JX434640), 91998. Herrera: Montuoso Forest Reserve, 7°44'05" N, 80°47'55" W (27), 500 m: SMF 91999-92001.
Comparative sequences from Genbank: ***Bolitoglossa colonnea***: Panama: Bocas del Toro: Finca de Enrique Quintero, Frente a Boca de Río Culubre, Río Changuinola, Distrito de Changuinola, 9°08'31" N, 82°30'05" W (12), 110-260 m: CH 6526 (FJ784318). Chiriquí: La Fortuna Forest Reserve (vicinity of 4): no voucher (AY526119). ***Bolitoglossa lignicolor***: Costa Rica: Puntarenas: Osa Peninsula, Buenos Aires: MVZ 11132 (AF218484). ***Bolitoglossa mexicana***: Mexico: Chiapas: 11 km E Belisario Domínguez: MVZ 176838 (GU725457). ***Bolitoglossa mombachoensis***: Nicaragua: Granada: Volcan Mombacho: SMF 78718 (AY133488). ***Bolitoglossa schizodactyla***: Panama: Coclé: Parque Nacional G. D. Omar Torrijos H. El Cope, 8°40'01" N, 80°35'31" W, 800 m: field number KRL 1179 (FJ784482), no voucher (AY526133).